Currently Pending Claims

1. A method for purifying alphavirus replicon particles comprising:

a) contacting a preparation containing alphavirus replicon particles with a tentacle ion exchange resin, under conditions and for a time sufficient to bind to said resin;

b) removing the portion of the preparation which is not bound to said ion exchange resin from said ion exchange resin;

c) eluting the bound alphavirus replicon particles from said ion exchange resin; and

d) recovering said replicon particles.

2. The method according to claim 1 wherein said tentacle ion exchange resin is a cationic exchange resin.

3. The method according to claim I wherein said tentacle ion exchange resin is an anionic exchange resin.

4. A method for purifying alphavirus replicon particles comprising at least two chromatographic purification steps wherein said purification steps are selected from the group consisting of ion exchange chromatography, size exclusion chromatography, hydrophobic interaction chromatography, affinity chromatography.

5. The method according to claim 4 wherein said ion exchange chromatography is performed using a tentacle ion exchange resin.

6. The method according to claim 4 wherein a first purification step is ion exchange chromatography and a second purification step is size exclusion chromatography.

7. An alphavirus replicon particle preparation made according to any one of claims 4-6.

8. An alphavirus replicon particle preparation according to claim 7 wherein said preparation comprises an immunogenic composition capable of expressing an antigen derived from a pathogenic agent.

9. The alphavirus replicon particle preparation according to claim 8 wherein said pathogenic agent is selected from the group consisting of viruses, bacteria, fungi, parasites, and cancerous cells.

10. An alphavirus replicon particle preparation according to claim 7 wherein said alphavirus replicon particle preparation comprises a therapeutic.

11. An alphavirus replicon particle preparation according to claim 1 0 wherein said alphavirus replicon particle preparation expresses a lymphokine, cytokine, or chemokine.

12. An alphavirus replicon particle preparation according to claim 7 wherein said lymphokine, cytokine or chemokine is selected from the group consisting of IL-2, IL-10, IL-
12, gamma interferon, GM-CSF, macrophage inflammatory protein (MIP)3α, MIP3β, and secondary lymphoid tissue chemokine (SLC).

13. A method for stimulating an immune response within a warm-blooded animal, comprising administering to a warm-blooded animal the alphavirus replicon particle preparation of claim 7.

14. The method according to claim 13 wherein said alphavirus replicon particle preparation expresses a lymphokine, cytokine, or chemokine.

15. The method according to claim 14 wherein said lymphokine, cytokine or chemokine is selected from the group consisting of IL-2, IL-10, IL-12, gamma interferon,GM-CSF, macrophage inflammatory protein (MIP)3α, MIP3β, and secondary lymphoid tissue chemokine (SLC).

16. A method of producing alphavirus replicon particles comprising:
a) infecting alphavirus packaging cells with a seed stock of alphavirus replicon particles;
b) incubating the infected packaging cells in a bioreactor, under conditions and for a time sufficient to permit the production of alphavirus replicon particles; and
c) harvesting culture supernatants containing said replicon particles.

17. A method,according to claim 16 wherein said bioreactor is an external component bioreactor.

18. A method according to claim 16 wherein said bioreactor is a suspension culture bioreactor.

19. A method according to claim 16 wherein said bioreactor is a hollow fiber bioreactor.

20. A method for producing alphavirus replicon particles comprising:
a) transfecting alphavirus packaging cells with a DNA-based alphavirus replicon or eukaryotic layered vector initiation system;
b) incubating the transfected packaging cells in a bioreactor, under conditions and for a time sufficient to permit the production of alphavirus replicon particles; and
c) harvesting culture supernatants containing said replicon particles.

21. A method for producing alphavirus replicon particles comprising:

a) transfecting alphavirus packaging cells with an alphavirus RNA vector replicon transcribed in vitro;

b) incubating the transfected packaging cells in a bioreactor, under conditions and for a time sufficient to permit the production of alphavirus replicon particles; and

c) harvesting culture supernatants containing said replicon particles.

22. A method for detecting multiple recombination events in a population of alphavirus replicon particles comprising:

a) providing a nucleic acid substrate suitable for detecting multiple recombination events, said substrate derived from said population of alphavirus replicon particles;

b) reacting said nucleic acid substrate with at least one first reaction mixture comprising an oligonucleotide complementary to an alphavirus nonstructural protein gene and an oligonucleotide complementary to an alphavirus structural protein gene, wherein said structural protein is either a capsid protein or a non-capsid structural protein, under conditions suitable and for a time sufficient to permit amplification of said nucleic acid substrate to form a first reaction product;

c) reacting said first reaction product with a second reaction mixture comprising a oligonucleotide complementary to an alphavirus capsid protein gene and a oligonucleotide complementary to a non-capsid alphavirus structural protein gene, under conditions suitable and for a time sufficient to permit amplification of said nucleic acid template to form a second reaction product; and

d) determining the presence or absence of said second reaction product.

23. A method for detecting multiple recombination events in a population of alphavirus replicon particles comprising:

a) providing a nucleic acid substrate suitable for detecting multiple recombination events, said substrate derived from said population of alphavirus replicon particles,

b) reacting said nucleic acid substrate with a first reaction mixture comprising an oligonucleotide complementary to an alphavirus nonstructural protein gene and an oligonucleotide complementary to an alphavirus capsid protein gene, under conditions suitable and for a time sufficient to permit amplification of said nucleic acid substrate to form a first reaction product;

c) reacting said first reaction product with a second reaction mixture comprising a oligonucleotide complementary to an alphavirus capsid protein gene and a oligonucleotide complementary to a non-capsid alphavirus structural protein gene, under conditions suitable and for a time sufficient to permit amplification of said nucleic acid template to form a second reaction product; and

d) determining the presence or absence of said second reaction product.

24. A method for detecting multiple recombination events in a population of alphavirus replicon particles comprising:

a) providing a nucleic acid substrate suitable for detecting multiple recombination events, said substrate derived from said population of alphavirus replicon particles;

b) reacting said nucleic acid substrate with a first reaction mixture comprising an oligonucleotide complementary to an alphavirus nonstructural protein gene and an oligonucleotide complementary to a non-capsid alphavirus structural protein gene, under conditions suitable and for a time sufficient to permit amplification of said nucleic acid substrate to form a first reaction product,

c) reacting said first reaction product with a second reaction mixture comprising a oligonucleotide complementary to an alphavirus capsid protein gene and a oligonucleotide complementary to a non-capsid alphavirus structural protein gene, under conditions suitable and for a time sufficient to permit amplification of said nucleic acid template to form a second reaction product; and

d) determining the presence or absence of said second reaction product.

25. A method for quantitating alphavirus replicon vector particles comprising:

a) providing a population of packaging cells;

b) contacting said packaging cells with said alphavirus replicon vector particles under conditions suitable and for a time sufficient for said cells to be infected with said alphavirus replicon vector particles;

c) incubating said infected packaging cells under conditions suitable and for a time sufficient for production of said alphavirus replicon vector particle;

d) enumerating the number of resulting plaques.

26. The method according to claim 25, wherein said packaging cells express all structural proteins necessary for packaging of said alphavirus replicon vector particles.

27. The method according to claim 25, wherein said packaging cells comprise at least one expression cassette expressing an alphavirus capsid protein and at least one alphavirus glycoprotein.

28. The method according to claim 25, wherein said packaging cells express an alphavirus capsid protein and at least one alphavirus glycoprotein from distinct expression cassettes.

29. (New) The method of claim 27, wherein said at least one expression cassette expresses E1 and E2 glycoproteins.

30. (New) The method of claim 25, further comprising the step of overlaying said infected cells with a layer of agarose.

31. (New) A composition comprising quantified alphavirus replicon particles produced by the method of claim 25.